

Quantikine[®]

Mouse/Rat PDGF-BB Immunoassay

Catalog Number MBB00

For the quantitative determination of mouse or rat Platelet-Derived Growth Factor BB (PDGF-BB) concentrations in cell culture supernates, serum and plasma.

This package insert must be read in its entirety before using this product.

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

TABLE OF CONTENTS

Contents	Page
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	3
LIMITATIONS OF THE PROCEDURE	3
PRECAUTION	3
TECHNICAL HINTS	3
REAGENTS	4
STORAGE	4
OTHER SUPPLIES REQUIRED	5
SAMPLE COLLECTION AND STORAGE	5
SAMPLE PREPARATION	5
REAGENT PREPARATION	6
ASSAY PROCEDURE	7
PROCEDURE SUMMARY AND CHECKLIST	8
CALCULATION OF RESULTS.	9
TYPICAL DATA	9
PRECISION.	10
RECOVERY	10
LINEARITY	11
SENSITIVITY	11
CALIBRATION	12
SAMPLE VALUES	12
SPECIFICITY	13
REFERENCES	14
PLATE LAYOUT	15

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INTRODUCTION

The Platelet-Derived Growth Factor (PDGF) family consists of proteins derived from four genes (PDGF-A, -B, -C and -D) that form four disulfide-linked homodimers (PDGF-AA, -BB, -CC, -DD) and one heterodimer (PDGF-AB) (1 - 3). These proteins and the related VEGF family proteins share the conserved PDGF/VEGF homology domain characterized by a pattern of highly conserved cysteine residues, which form the cysteine knot motif. PDGF-B is synthesized as a pre-pro-protein, which has a signal peptide, a mature region, an N-terminal pro-peptide, and a C-terminal extension. The C-terminal extension contains a conserved cell retention motif that confers retention of the secreted PDGF-BB within the pericellular space (4 - 6). Most cells produce both PDGF-A and B chains. The individual chains are assembled stochastically into disulfide-linked inactive homodimeric or heterodimeric precursors in the endoplasmic reticulum (1 - 3). Within the trans-Golgi network, these precursors then undergo the intracellular proteolytic processing necessary for the secretion of the biologically active mature proteins. PDGF-A and -B isoforms were originally isolated from platelets, but were subsequently found to be produced by multiple cell types including megakaryocytes, fibroblasts, keratinocytes, vascular smooth muscle cells, endothelial cells, neurons, Schwann cells, and macrophages (3). The mature mouse PDGF-B chain shares 98%, 89%, and 88% amino acid sequence identity to that of rat (7), human (4), and dog (8), respectively. The mature mouse A and B chains share approximately 57% amino acid sequence homology (6, 9).

PDGF family proteins regulate diverse cellular functions by binding to and inducing the homo- or heterodimerization of two receptor subunits (PDGF R α and R β) (1 - 3). Both subunits belong to the class III subfamily of receptor tyrosine kinases. PDGF-BB can induce α/α or β/β homodimerization as well as α/β heterodimerization. PDGF plays important roles in development (10) and regeneration. The major source of PDGF in blood is from platelets, which releases PDGF into circulation upon platelet activation.

The Quantikine Mouse/Rat PDGF-BB Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse/rat PDGF-BB in cell culture supernates, serum and plasma. It contains *E. coli*-expressed recombinant rat PDGF-BB and antibodies raised against recombinant human PDGF-BB. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse/rat PDGF-BB showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine Mouse/Rat PDGF-BB kit can be used to determine relative mass values for naturally occurring mouse/rat PDGF-BB.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-human PDGF-BB monoclonal antibody has been pre-coated onto a microplate. Standards, controls, and samples are pipetted into the wells and any PDGF-BB present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal goat anti-human PDGF-BB antibody is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of PDGF-BB bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other sources or lots.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested, however, the possibility of interference cannot be excluded.

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.

REAGENTS

Mouse/Rat PDGF-BB Microplate (Part 892860) - One 96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody specific for PDGF-BB.

Mouse/Rat PDGF-BB Conjugate (Part 892861) - 12.5 mL of a polyclonal antibody conjugated to horseradish peroxidase with preservatives.

Mouse/Rat PDGF-BB Standard (Part 892862) - 2 vials (10 ng/vial) of recombinant rat PDGF-BB in a buffered protein base with preservatives, lyophilized.

Mouse/Rat PDGF-BB Control (Part 892863) - 1 vial of recombinant rat PDGF-BB in a buffered protein base with preservatives, lyophilized. The concentration range of rat PDGF-BB after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.

Assay Diluent RD1X (Part 895121) - 11 mL of a buffered protein solution with preservatives.

Calibrator Diluent RD5-3 (Part 895436) - 21 mL of a buffered protein solution with preservatives.
For cell culture supernate samples.

Calibrator Diluent RD6-3 (Part 895165) - 21 mL of animal serum with preservatives.
For serum/plasma samples.

Wash Buffer Concentrate (Part 895024) - 50 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895174) - 23 mL of a diluted hydrochloric acid.

Plate Covers (Part 640197) - 4 adhesive strips.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Mouse/Rat PDGF-BB Conjugate	May be stored for up to 1 month at 2 - 8° C.*
	Diluted Wash Buffer	
	Stop Solution	
	Assay Diluent RD1X	
	Calibrator Diluent RD5-3	
	Calibrator Diluent RD6-3	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Mouse/Rat PDGF-BB Standard (2000 pg/mL)	
	Mouse/Rat PDGF-BB Control	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 1000 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell culture supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature or overnight at $2 - 8^{\circ}$ C before centrifuging for 20 minutes at 2000 x g for mouse blood and 1000 x g for rat blood. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge at $2 - 8^{\circ}$ C for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes at $2 - 8^{\circ}$ C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

PDGF is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of PDGF, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

SAMPLE PREPARATION

Rat serum samples require a 2-fold dilution into Calibrator Diluent RD6-3 prior to assay. A suggested 2-fold dilution is 70 μ L sample + 70 μ L Calibrator Diluent RD6-3.

Rat EDTA and heparin plasma samples do not require dilution.

Mouse serum samples require a 10-fold dilution into Calibrator Diluent RD6-3 prior to assay. A suggested 10-fold dilution is 20 μ L sample + 180 μ L Calibrator Diluent RD6-3.

Mouse EDTA plasma samples require a 5-fold dilution into Calibrator Diluent RD6-3 prior to assay. A suggested 5-fold dilution is 40 μ L sample + 160 μ L Calibrator Diluent RD6-3.

Mouse heparin plasma samples require a 2-fold dilution into Calibrator Diluent RD6-3 prior to assay. A suggested 2-fold dilution is 70 μ L sample + 70 μ L Calibrator Diluent RD6-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

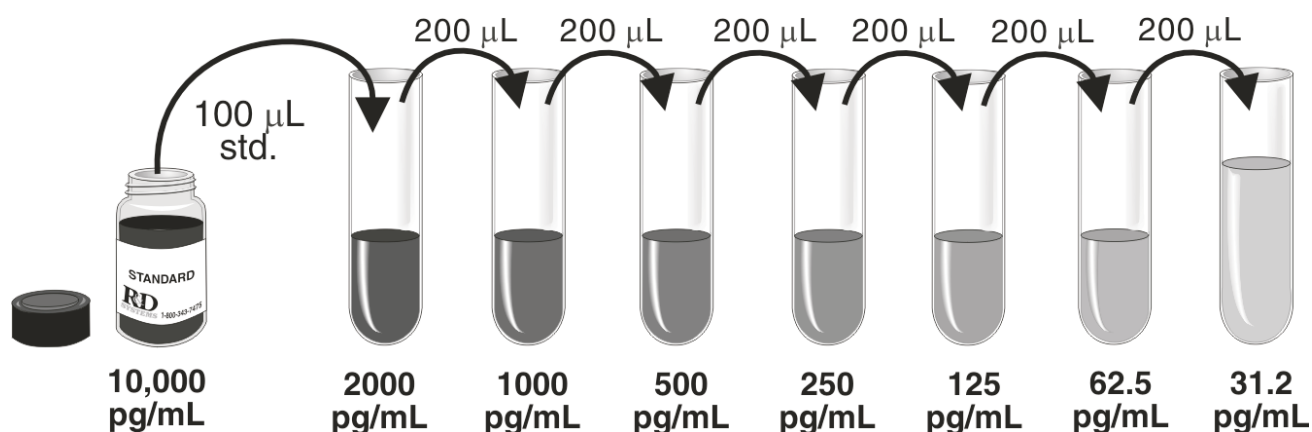
Mouse/Rat PDGF-BB Kit Control - Reconstitute the Kit Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 25 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 625 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 μ L of the resultant mixture is required per well.

Mouse/Rat PDGF-BB Standard - Reconstitute the Mouse/Rat PDGF-BB Standard with 1.0 mL of Calibrator Diluent RD5-3 (*for cell culture supernate samples*) or Calibrator Diluent RD6-3 (*for serum/plasma samples*). Do not substitute other diluents. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 400 μ L of the appropriate Calibrator Diluent (*Calibrator Diluent RD5-3 for cell culture supernate samples or Calibrator Diluent RD6-3 for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 200 μ L of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, standard dilutions, controls and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 50 μL of Assay Diluent RD1X to each well.
4. Add 50 μL of Standard, Control or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 μL of Mouse/Rat PDGF-BB Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Serum and plasma samples require dilution. See Sample Preparation section.

PROCEDURE SUMMARY AND CHECKLIST

1. Bring all reagents to room temperature.
 Prepare reagents and samples as instructed.
 Return unused components to storage temperature as indicated in the instructions.
2. Add 50 μ L Assay Diluent to each well.
3. Add 50 μ L Standard, Control, or sample* to each well.
 Tap plate gently for one minute.
 Cover the plate and incubate for 2 hours at room temperature.
4. Aspirate and wash each well five times.
5. Add 100 μ L Conjugate to each well.
 Cover the plate and incubate for 2 hours at room temperature.
6. Aspirate and wash each well five times.
7. Add 100 μ L Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
8. Add 100 μ L Stop Solution to each well.
9. Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).

*Serum and plasma samples require dilution. See Sample Preparation.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

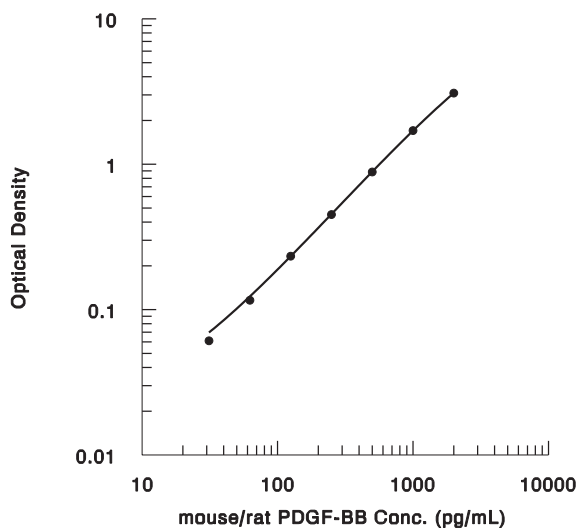
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse/rat PDGF-BB concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Because serum and plasma samples have been diluted prior to assay, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

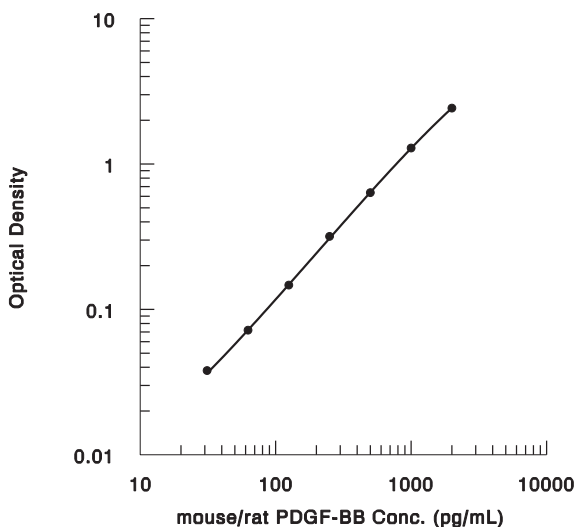
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Calibrator Diluent RD5-3



pg/mL	O.D.	Average	Corrected
0	0.068	0.070	—
	0.071		
31.2	0.129	0.131	0.061
	0.132		
62.5	0.184	0.186	0.116
	0.187		
125	0.301	0.303	0.233
	0.304		
250	0.516	0.520	0.450
	0.524		
500	0.937	0.954	0.884
	0.971		
1000	1.753	1.775	1.705
	1.796		
2000	3.101	3.158	3.088
	3.214		

Calibrator Diluent RD6-3



pg/mL	O.D.	Average	Corrected
0	0.072	0.076	—
	0.079		
	0.112		
31.2	0.114	0.113	0.037
	0.145		
	0.149		
62.5	0.217	0.147	0.071
	0.228		
	0.228		
125	0.390	0.223	0.147
	0.397		
	0.397		
250	0.704	0.394	0.318
	0.718		
	0.718		
500	1.359	0.711	0.635
	1.369		
	1.369		
1000	2.500	1.364	1.288
	2.500		
	2.500		
2000	2.503	2.502	2.426
	2.503		

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in at least twenty-two separate assays to assess inter-assay precision.

Cell Culture Supernate Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	22	24	24
Mean (pg/mL)	49.7	252	823	52.1	246	811
Standard deviation	4.5	18.7	36.7	5.3	21.1	40.4
CV (%)	9.0	7.4	4.5	10.2	8.6	5.0

Serum/Plasma Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	22	22	23
Mean (pg/mL)	61.1	298	1019	69.5	305	1048
Standard deviation	5.6	24.6	40.3	6.6	26.3	98.5
CV (%)	9.2	8.3	4.0	9.5	8.6	9.4

RECOVERY

The recovery of rat PDGF-BB spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range (%)
Mouse cell culture supernates (n=8)	100	89 - 118
Rat cell culture supernates (n=8)	102	94 - 114
Rat serum* (n=5)	100	89 - 113
Mouse heparin plasma* (n=5)	96	85 - 113
Rat EDTA plasma (n=5)	102	86 - 111
Rat heparin plasma (n=5)	102	85 - 111

*Samples were diluted prior to assay as described in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples spiked with or containing PDGF-BB in each matrix were diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Mouse cell culture supernates (n=5)	Mouse serum* (n=5)	Mouse EDTA plasma* (n=5)	Mouse heparin plasma* (n=5)
1:2	Average % of Expected	96	94	95	93
	Range (%)	86 - 102	93 - 96	93 - 97	89 - 97
1:4	Average % of Expected	102	94	92	91
	Range (%)	96 - 105	89 - 101	87 - 99	84 - 97
1:8	Average % of Expected	106	98	91	93
	Range (%)	97 - 111	93 - 104	84 - 102	85 - 106
1:16	Average % of Expected	103	90	93	94
	Range (%)	93 - 113	84 - 97	83 - 106	81 - 108

		Rat cell culture supernates (n=5)	Rat serum* (n=5)	Rat EDTA plasma (n=5)	Rat heparin plasma (n=5)
1:2	Average % of Expected	98	94	95	94
	Range (%)	90 - 110	89 - 97	91 - 97	87 - 99
1:4	Average % of Expected	98	92	91	95
	Range (%)	93 - 108	90 - 94	88 - 95	87 - 99
1:8	Average % of Expected	99	93	93	98
	Range (%)	92 - 112	89 - 104	89 - 97	92 - 109
1:16	Average % of Expected	98	92	94	99
	Range (%)	90 - 112	86 - 100	84 - 103	81 - 112

*Samples were diluted as described in the Sample Preparation section.

SENSITIVITY

Thirty-six assays were evaluated and the minimum detectable dose (MDD) of mouse/rat PDGF-BB ranged from 4.0 - 19.3 pg/mL. The mean MDD was 7.7 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant rat PDGF-BB produced at R&D Systems. The disulfide-linked homodimeric recombinant rat PDGF-BB, containing two 109 amino acid residue B chain monomers, has a predicted molecular mass of approximately 25 kDa.

The protein concentration of the recombinant rat PDGF-BB was determined by the method of Bradford (11) using purified bovine serum albumin as a standard.

SAMPLE VALUES

Serum/plasma - Individual mouse and rat serum and plasma samples were evaluated for detectable levels of PDGF-BB in the assay.

Sample	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Mouse serum* (n=10)	12,830	10,270 - 17,390	2148
Mouse EDTA plasma* (n=10)	4237	2066 - 8210	2251
Mouse heparin plasma* (n=10)	1045	351 - 1584	379
Rat serum* (n=10)	2684	2054 - 3576	514
Rat EDTA plasma (n=10)	978	506 - 1551	442.6
Rat heparin plasma (n=10)	1018	218 - 1548	485.5

*Samples were diluted prior to assay as described in the Sample Preparation section.

Cell culture supernates - The mouse macrophage cell line, J774A.1 (1×10^6 cells/mL), was cultured for 4 days in 50 mL DMEM supplemented with 10% fetal calf serum, stimulated with 2.5 ng/mL of LPS. The cell culture supernate was evaluated for detectable levels of PDGF-BB and measured 3092 pg/mL.

SPECIFICITY

This assay recognizes both recombinant and natural mouse and rat PDGF-BB. The factors listed below were prepared at 50 ng/mL in the appropriate Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse/rat PDGF-BB control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	M-CSF	Recombinant rat:	Recombinant human:	Recombinant porcine:
FGF-8b	P/IGF-2	GM-CSF	PDGF-AA	GM-CSF
FGF-8c	VEGF ₁₂₀	β -NGF	PDGF-C	
Flt-3 Ligand	VEGF ₁₆₄	PDGF-AA	PDGF R α	Others:
G-CSF	VEGF-D	VEGF ₁₆₄	PDGF R β	bovine FGF acidic
GM-CSF	VEGF R1/Fc			bovine FGF basic
IGF-I	VEGF R2/Fc			
IGF-II	VEGF R3/Fc			

Some cross-reactivity was observed with the following:

Factor	Concentration Tested (pg/mL)	% Cross-reactivity
Recombinant rat PDGF-AB	2000	10.2
Recombinant human PDGF-AB	2000	5.3
Recombinant human PDGF-BB	2000	64.7
Natural human PDGF	2000	38.1
Natural porcine PDGF	2000	65.2

Some interference was observed with the following:

Recombinant mouse PDGF R α	At concentrations greater than 10,000 pg/mL
Recombinant mouse PDGF R β	At concentrations greater than 1000 pg/mL
Recombinant rat PDGF R β	At concentrations greater than 5000 pg/mL

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PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								